

dextrane. Virus yield was reduced to about 2% of the controls (line 2, 13–16). If RNA-synthesis is inhibited by the addition of Actinomycin D, the inhibition of virus growth by poly(rI)·poly(rC) is prevented and the virus yield is the same or even enhanced compared to control cultures (line 4, 17–20). These findings are similar to those obtained previously with *Sindbis* Virus and suggest that poly(rI)·poly(rC) exerts its effect by inducing the formation of one or more intermediate substances, which are responsible for the growth inhibition of *Semliki Fores* Virus<sup>3, 4</sup>. One possibility which cannot be excluded though is that

poly(rI)·poly(rC) inhibits virus growth not by inducing specifically an antiviral substance but rather because of an unspecific toxic effect on the host cell, which is not appearing in Actinomycin D treated cells. Since it has been shown that doublestranded polynucleotides can induce interferon this type of protein is one of the possible candidates for an induced intermediate substance. Besides the possibility of additional sofar not detected antiviral substances in poly(rI)·poly(rC) treated cells the presence of interferon could secondarily result in the induction of other cellular antiviral proteins.

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### Biosynthesis of Juvenile Hormone from 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic Acid in the Adult *Cecropia* Moth

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The biosynthesis of juvenile hormone (JH, **1**) and its lower homologue (JH-II, **2**) in the adult silkmoth *Hyalophora cecropia* (L.) involves L-methionine, which provides the ester methyl group for both compounds<sup>1</sup>. The substrate for the methylation reaction, however, has not yet been identified. We now wish to report that adult male *H. cecropia* are able to synthesize JH from *dl-trans,trans,cis*-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic acid (**3**). For the preparation of labelled **3** we took advantage of the fact that JH, when injected into late fifth instar larvae of the tobacco hornworm, *Manduca sexta* (JOHANSSON), is rapidly inactivated

by enzymatic hydrolysis to **3**<sup>2</sup>. Labelled **3** was most conveniently prepared by *in vitro* incubation of 2-<sup>14</sup>C-*dl*-JH with *Manduca* blood.

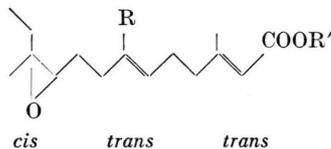
#### Materials and Methods

2-<sup>14</sup>C-*dl*-JH<sup>3</sup> (5.0·10<sup>6</sup> dpm, spec. act. 25 mCi/mmole) was incubated at 37° with 2 ml hemolymph of 13–19 day-old last instar larvae of *M. sexta*. After 2 hrs. the mixture was extracted four times with 2 ml ether–ethanol (2:1) and the extract resolved by thin layer chromatography on silica gel with hexane–ethyl acetate (7:3). The epoxy acid on the TLC-plate (*R<sub>f</sub>* ~0.3) was located with a radio scanner and eluted with peroxide free tetrahydrofuran (**3**; 4.1·10<sup>6</sup> dpm, 82%). From a second zone (*R<sub>f</sub>* ~0.8) unchanged JH was recovered (**1**; 0.87·10<sup>6</sup> dpm, 17%).

The epoxy acid **3** was dissolved in TRIS/HCL buffer pH 8.5 just prior to application and injected (3 male *Cecropia* moths, 2 days old, 50 μl/moth) through the membrane between the 6th and 7th abdominal segments. In a second experiment [methyl-<sup>3</sup>H]-L-methionine (2.6 Ci/mmole, Schwarz Bioresearch Inc. N. Y., in Insect-Ringer solution, 30 μl/moth) was administered together with **3**. The other experimental conditions and the isolation procedure for JH and JH-II were the same as previously described<sup>1, 4</sup>.

#### Results and Discussion

Five hours after injection of the racemic 2-<sup>14</sup>C-epoxy acid **3** into 2 day-old male *Cecropia* moths the JH was isolated. It contained 3.9% of the administered label (Table I, experiment 1). In a second experiment [methyl-<sup>3</sup>H]-L-methionine was injected simultaneously with 2-<sup>14</sup>C-**3** and the incubation period extended to 15 hours (Table I, experiment 2). In this case 5.8% of the <sup>14</sup>C and 0.11% of the <sup>3</sup>H was recovered with the JH. The purity of the isolated JH was checked through catalytic hydrogenation: the reaction product methyl 3,11-dimethyl-7-ethyl-tridecanoate<sup>5</sup> after gas chromatographic



	<i>cis</i>	<i>trans</i>	<i>trans</i>
<b>1</b>	R = -C <sub>2</sub> H <sub>5</sub>	R' = -CH <sub>3</sub>	
<b>2</b>	R = -CH <sub>3</sub>	R' = -CH <sub>3</sub>	
<b>3</b>	R = -C <sub>2</sub> H <sub>5</sub>	R' = -H	

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purification<sup>1</sup> had the same dpm <sup>3</sup>H/dpm <sup>14</sup>C ratio (2.39 : 1) as the starting material (2.43 : 1). In the second experiment also JH-II was isolated; it contained <sup>3</sup>H but no detectable <sup>14</sup>C activity. Exogenous

	Experi- ment 1	Experi- ment 2
2- <sup>14</sup> C-epoxy acid <b>3</b> , dose/moth [dpm]	430 × 10 <sup>3</sup>	350 × 10 <sup>3</sup>
[methyl- <sup>3</sup> H]-L-methionine, dose/moth [dpm]	—	55 × 10 <sup>6</sup>
incubation period [hrs.]	5	15
JH isolated per moth [μg]	1.4	1.8
[dpm <sup>14</sup> C]	16.7 × 10 <sup>3</sup>	20.2 × 10 <sup>3</sup>
[dpm <sup>3</sup> H]	—	62.2 × 10 <sup>3</sup>
JH-II isolated per moth [μg]	—	0.4
[dpm <sup>14</sup> C]	—	0
[dpm <sup>3</sup> H]	—	11.3 × 10 <sup>3</sup>

Table I. Incorporation-experiments with 2-<sup>14</sup>C-*dl-trans,trans*, *cis*-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic acid and [methyl-<sup>3</sup>H]-L-methionine. Three 2 day-old male *Cecropia* moths were used in each experiment.

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JH administered to adult *H. cecropia* is rapidly metabolized, and after 24 hours only 10% of the hormone could be recovered unchanged (unpublished results). In view of the high rate of incorporation of the acid **3** into JH — ~10% after 15 hours, assuming a JH recovery of ~50% — it appears unlikely that the inactivation of JH in this animal proceeds primarily through hydrolysis to the acid **3**. On the other hand, **3** is not necessarily a natural precursor in the biosynthesis of JH. Conceivably another acidic compound is methylated and afterwards, perhaps by epoxidation, converted to the JH. Experiments with JH have shown that its biological activity depends largely on the carrier with which it is administered<sup>6</sup>. These results may be explained by the differing degrees of protection afforded by the carriers in the presence of degrading enzyme systems. It is also possible that exogenous JH is not degraded through the same metabolic pathways as endogenous JH.

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## Fish Eggs: Another New Source of Polyvalent Proteinase-Inhibitors

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It has recently been demonstrated<sup>1</sup> that the albumin gland of snails not only supports the snail eggs with anti-bacterial and blood or tumor cell crossreacting agglutinins (protectins)<sup>2</sup>, but also with large amounts of polyvalent proteinase-inhibitors, several of which could be detected by the method of fibrin-agar-electrophoresis in a great number of different and quite unrelated snail eggs<sup>3, 4</sup>.

Subsequently we tested some fish eggs for the presence of proteinase-inhibitors, as the occurrence of heterophile agglutinins in certain fish eggs had already been described following the discovery of agglutinins in snail eggs<sup>2</sup>. The importance of these egg proteinase-inhibitors and agglutinins is not very well understood. It may be that they have a protective function towards bacterial invasion or that they are used in order to neutralize the sperm penetrating protease acrosin<sup>5, 6</sup>. The fact that proteinase-inhibitors are also known to occur in chicken, turkey and other avian eggs (avian egg white inhibitors)<sup>7</sup> points out that we are dealing here with a wide spread biological phenomenon, which offers itself for clinical investigations and awaits further practical applications<sup>8</sup>.

Fibrin-agar-electrophoresis was performed as described in detail previously<sup>3, 4</sup>, where also the source of the proteolytic enzymes is given. Fresh or frozen

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